Agrobacterium tumefaciens-infection Strategies for Greater Transgenic Recovery in Nicotiana tabacum cv. TAPM26

Abstract

The efficiency of vir gene induction and conditions of T-DNA delivery are important parameters for developing an efficient Agrobacterium-mediated plant transformation system. Reliable selection regimes coupled with an efficient regeneration method are important for obtaining stably transformed plants. This study reported a modified protocol for greater recovery of transgenic tobacco (Nicotiana tabacum cv. TAPM 26) plant using Agrobacterium tumefaciens-mediated transformation combined with herbicide Basta as the selection agent. Effective post co-cultivation conditions were developed by investigating the selection regime in vitro and ex vitro. The effects of carbenicillin on Agrobacterium growth and plant regeneration to determine non-phytotoxic concentration of carbenicillin was also evaluated as antibiotics used for Agrobacterium growth suppression have known to adversely affect plant regeneration. It was discovered that the efficient conditions that led to greater recovery of transformants includes the exposure to 400 mg L-1 of carbenicillin post-co-cultivation for Agrobacterium elimination, 1 week of recovery period prior to Basta selection and prolonged selection pressure extended to rooting process which significantly reduces chimeric events. Basta concentration of 1.5 mg L-1 at tissue culture level was most effective in maintaining selection pressure without affecting the plants' regeneration competency. Using this improved protocol, an average of 7.00±0.33 Basta resistant shoots were obtained per leaf explant transformed and a total of 371 independently transformed tobacco plants were regenerated in this study. This highly reproducible protocol could be used to produce transgenic tobacco plants expressing useful traits or can be utilized as model system to study the expression of particular genes.